Effects of Hypoxia on the Development of Intestinal Enzymes in Neonatal and Juvenile Rats

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Hypoxia in the neonate is known to alter the activity of hepatic and pancreatic enzymes involved in lipid and carbohydrate metabolism. The purpose of this study was to evaluate the effect of neonatal hypoxia on the activity of intestinal enzymes, and to determine whether the administration of glucocorticoids to neonates can mimic the effects of hypoxia. Hypoxia in neonatal rats (0–7 days) increased protein content, and lactase and maltase activity in the duodenal and the jejunal segments of the small intestine compared with normoxic controls. Hypoxia in juvenile rats (28–35 days) did not change these enzymes. Two weeks after returning hypoxic (0–7 days) pups to normoxia, their body weight remained lower than the age-matched controls. In the group recovering from hypoxia, sucrase, maltase, and leucine aminopeptidase activities were lower in the duodenal and the jejunal segment. Compared with controls, LDH activity was lower only in the jejunal intestine in the group recovering from hypoxia. All enzyme activities returned to control levels 3 weeks after recovery. Neonatal rats treated with dexamethasone had a decrease in body weight, but increases in sucrase and maltase activity in both the duodenal and the jejunal segment. Hypoxia in newborn rats caused a delayed maturation of small intestinal enzymes. Increases in serum glucocorticoids after hypoxic exposure probably do not play a major role in the delayed maturation of the disaccharidase activity in the small intestine. Exp Biol Med 228:717–723, 2003

Key words: hypoxia; intestinal enzymes; development

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Neonatal hypoxia occurs frequently and is usually accompanied by serious results in the affected infant (1, 2). Although considerable efforts have been directed at evaluating alterations in neurological, cardiopulmonary, and renal function (3, 4), the digestive function in response to hypoxia in the neonate has not been fully evaluated. We have shown recently that hypoxia in neonatal rats caused dysfunction of lipid metabolism partly due to interference with hepatic lipase development (5). Similar effects of hypoxia may also occur in the small intestine. In neonatal rats, as in human infants, the intestine undergoes rapid differentiation and maturation of its digestive enzymes (6–10). Neonates have high lactase, undetectable sucrase, and low maltase activity. In rodents, sucrase and maltase activity increase rapidly around the 15th day of age. These enzymes continue to increase until they reach adult levels after weaning. Lactase, in contrast, starts to decrease rapidly as the animal approaches weaning and then gradually decreases to a low adult level (7).

We recently demonstrated that hypoxia increased plasma glucocorticoids in the suckling neonatal rats, but not in weaned 35-day-old rats (11–13). Glucocorticoids are known to play important roles in the development of intestinal enzymes in rats and other animals (6, 14, 15). Thus, hypoxia might affect intestinal development directly or indirectly through alterations in circulating glucocorticoids. Changes in concentrations of intestinal enzymes have an important impact on the overall digestive capacity of the animal. Such changes may affect the nutritional status of the animal that, in turn, may affect the function of other systems, including neurological, cardiac, pulmonary, or renal.

The aims of the present study were to compare changes in small intestinal enzymes in neonatal suckling rats exposed to hypoxia for 7 days from birth compared with juvenile, weaned rats exposed to hypoxia for 7 days (from 28 to 35 days of age); to evaluate the time course of recovery in intestinal measurements, in particular, digestive enzymes of neonatal rats exposed to hypoxic conditions; and to de-

termine whether the hypoxic effect on intestinal measurements can be mimicked by glucocorticoid administration.

Materials and Methods

Animal Treatment. All animal protocols were approved by the Institutional Animal Care and Use Committees of the Medical College of Wisconsin and St. Luke's/Sinai Samaritan Medical Center. Timed pregnant Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were obtained at 14 days gestation and were maintained on a standard sodium diet (Richmond Standard no. 5001) and water *ad libitum* in a controlled environment (0600-1800 hr lights on). Parturition usually occurred on the afternoon of gestational Day 21 during which time rats were kept under observation.

Hypoxia from 0 to 7 Days of Age. As soon as a litter was completely delivered, the dam and her pups (24 litters; 8-10 pups, mixed sexes/litter) were immediately moved to an environment chamber and were exposed to normobaric normoxia (21% O_2; room air) or hypoxia (12% O_2) as described previously (11, 16, 17). The experimental day was at the end of 7 days of exposure of dams and their litters to either normoxia or hypoxia. We have previously shown that this exposure leads to arterial PO_2 levels in adults of about 50 to 55 torr with sustained respiratory alkalosis with metabolic compensation (16, 17). Lactating dams were maintained with their litters for 7 days in a normoxic or hypoxic environment (11, 18). Chambers were briefly opened on Day 4 to clean the cages. At 0800 hr of Day 7, five litters of rat pups each from the hypoxic and normoxic group were decapitated. Up to four pups from each litter were randomly selected, and their intestinal mucosa were collected.

The remaining hypoxic litters were allowed to recover by returning them to a normoxic environment with their age-matched normoxic counterparts. Seven days after return to normoxic environment, two more litters of each from the recovery and control normoxic were decapitated. Up to four pups from each litter were randomly selected and their intestinal mucosa were collected and processed as above. The remaining litters from each group were sacrificed 14 and 21 days after their return to normoxic environment and their intestinal mucosa were harvested. Weaning was at 21 days of age.

Hypoxia from 28 to 35 Days of Age. Male and female rats (n = 16) from several randomly assigned litters raised under normoxic conditions were weaned at 21 days of age. At 28 days of age, they were placed in chambers and exposed to normoxia or hypoxia for 7 days. All animals were sacrificed at 35 days of age (7 days of hypoxia) and mucosal scrapings were collected as described below.

Treatment with Dexamethason. Eighteen newborn rats were divided into three subgroups with six animals each and were given by daily intraperitoneal injection one of the following: dexamethasone (13 ng/g/day), the same volume of dimethyl sulfoxide (the solvent for dexamethasone), or no injection. Dexamethasone was chosen based on its potency as a glucocorticoid compared with corticosterone as we have used previously (5, 23). The dose of dexamethasone was selected to produce a concentration approximately equivalent to the concentration of corticosterone reached in the serum of hypoxic pups (11). All animals were sacrificed at 7 days of age and their intestinal mucosa were harvested as described above.

Tissue Collection. After bleeding, the entire intestine (from the pyloric end to the ileal-cecal junction) was removed and trimmed of fat and mesentery. The intestine was divided into two equal parts: the proximal and distal halves. An 8-cm-long segment was removed starting from the pyloric end (from the proximal half) and represented the duodenal segment, and another 8-cm piece (jejunal segment) was removed from the distal half also from the pyloric end. Both segments were split and gently wiped with tissue paper to remove the intestinal contents. The mucosa were scraped using a glass coverslip and were immediately frozen in dry ice. Tissues were stored frozen at −80°C until use.

Tissue Homogenization. For the preparation of mucosal homogenate for mucosal enzymes and protein measurements, the partially thawed mucosal scraping was homogenized in 100 vol of ice-cold water with a hand-held Potter-Elvehjem homogenizer using a Teflon pestle. The homogenate was used immediately for enzyme and protein assays.

Biochemical Measurements. Lactase, sucrase, and maltase were determined by the method of Dahlquist (19), using lactose, sucrose, and maltose as the corresponding substrates. Disaccharidase activity was expressed as micromoles of disaccharides hydrolyzed per minute per gram of protein.

Leucine aminopeptidase activity was determined by the method of Szasz (20), using L-leucine-*p*-nitroanilide as the substrate. Units of enzyme activity were expressed as nanomoles of nitroaniline released per minute per milligram of protein.

LDH was determined by the formation of NADH from NAD using sodium lactate as the substrate in a potassium phosphate buffer (100 m*M*, pH 7.5, with 133 m*M* NaCl and 0.66 m*M* $MgCl_2$). Enzyme activities were expressed as picomoles of NADH formed (increases in OD_{340}) per minute per milligram of protein at 37°C.

Protein concentration was determined by the method of Lowry (21) using bovine serum albumin fraction V as the standard.

Statistics. Results are reported as means ± SE. Student's t test was used to compare the means of two groups. Multiple-factor analysis of variance (ANOVA) was used to evaluate overall effects and interactions between factors. Each pup was considered to be one subject. Rather than pooling or nesting each litter for statistical analysis, we randomly selected up to four pups from multiple litters and considered each pup as n = 1. We have extensively used this statistical approach previously (5, 8, 11–13, 18, 23, 24).

Duncan's multiple range test was used to compare the means between two specific groups with $P \leq 0.05$ considered as significant.

Results

Hypoxia from birth to 7 days of age resulted in a decrease in body weight (8.7 ± 0.3 g) compared with normoxic control (14.2 ± 0.4 g). Hypoxia resulted in an increase in mucosal protein content in both duodenal and jejunal segments, with only the jejunal segment reaching statistical significance (duodenal segment – HP = 157.5 ± 2.7, C = 138.3 ± 11.8; jejunal segment – HP = 176.2 ± 3.3*, C = 157.5 ± 2.7 mg/gram tissue). The hypoxic group showed an increase in lactase activity in the jejunal segment of the small intestine (Fig. 1). In the duodenal segment, lactase tended to increase but did not reach statistical significance. Maltase showed an increase after hypoxia in both the duodenal and jejunal segments. Sucrase activity was undetectable in the 7-day-old rats. Table I summarizes the intestinal disaccharidase and protein measurements in juvenile rats weaned at 21 days of age and subsequently exposed to hypoxia from 28 to 35 days of age. There was no effect of hypoxia on body weight (not shown) and intestinal measurements in these rats.

Table II shows the body weight and intestinal mucosal measurements of rat pups exposed to hypoxia from birth to 7 days of age at specific times after their return to normoxic environment. When the hypoxic 7-day-old animals were returned to normoxic conditions for 7 days (14 days of age), their body weight was lower than the age-matched controls. Mucosal protein content was not different between the two groups. At 14 days after the return of the 7-day hypoxic animals to normoxia (21 days of age), their body weights remained lower than the control counterparts (Table II). Their intestinal mucosal protein contents were similar to controls. At 21 days after the return of the 7-day hypoxic animals to normoxic condition (28 days of age), their body weights were still lower than the controls, but mucosal protein content was similar to controls (Table II).

At 7 days of recovery (14 days of age), there was no difference in lactase and maltase between previously hypoxic and control groups (Fig. 2). There was no detectable sucrase in both the normoxic and hypoxic group. At 14 days of recovery (21 days of age), mucosal lactase activity was similar to control in the duodenal and jejunal segment. Sucrase and maltase activity were lower than controls in both the duodenal and jejunal segment (Fig. 2). At 21 days of recovery (28 days of age), sucrase and maltase had returned to the levels found in controls (Fig. 2).

To determine if the effect of prior hypoxia on sucrase and maltase activity in the 14-day recovery group (21 days of age) was also seen in other digestive enzymes, we measured leucine aminopeptidase, a marker protease in the

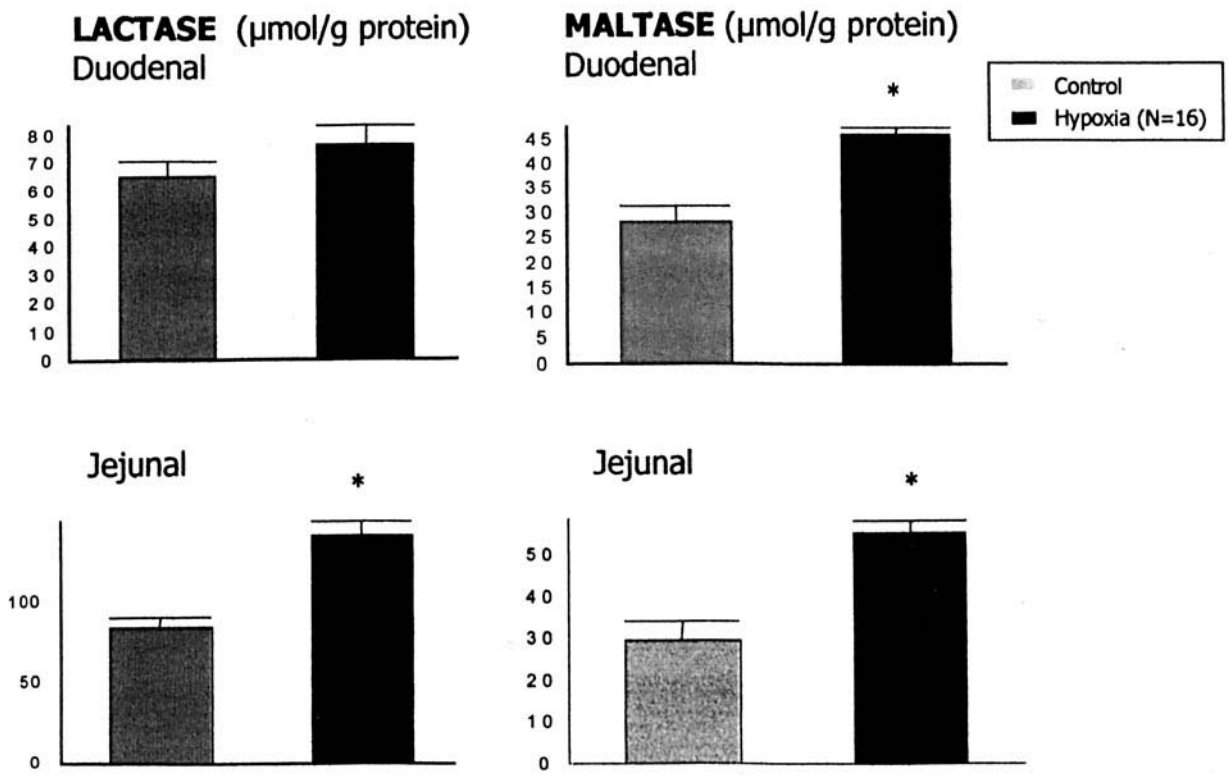

Figure 1. Comparison of intestinal lactase and maltase activity in duodenal and jejunal segments of 7-day-old rats exposed to hypoxia from 7 days (0-7 days of age). There were 16 animals in each group from five separate litters. All values are means ± SE. Shaded bars represent normoxic controls and solid bars represent hypoxic animals. * Indicates that values significantly different from corresponding control values with $P \leq 0.05$. The vertical lines represent SE of the corresponding means.

Table I. Comparison of Disaccharidase Activity and Protein Concentration in Juvenile Rats Exposed to Hypoxia for 7 Days (28–35 Days of Age)

Treatments	Intestinal segment	Protein content (mg/gm tissue)	Lactase	Sucrase	Maltase
			(μmol//min/g protein)		
Normoxic (8)	Duodenal	167.1 ± 2.8	5.3 ± 0.8	42.2 ± 4.0	136.0 ± 9.3
	Jejunal	173.4 ± 3.2	30.1 ± 2.1	117.2 ± 6.9	269.1 ± 12.2
Hypoxic (8)	Duodenal	154.1 ± 0.8	6.8 ± 1.2	41.1 ± 3.9	166.7 ± 24.4
	Jejunal	170.5 ± 2.5	33.0 ± 1.8	103.9 ± 5.6	266.7 ± 19.4

Note. All values are mean ± SE. (*n*) is the number of animals in each group.

Table II. Recovery of Body Weight and Intestinal Protein in 7 Day Hypoxic Rats after Return to Normoxic Environment

Age	Body weight (gm)	Intestinal segment	Protein content (mg/gm·tissue)
14 days old			
Normoxic (8)	25.6 ± 0.7	Duodenal	136.5 ± 1.9
		Jejunal	144.5 ± 2.8
7 day post-hypoxia (8)	19.7 ± 0.5*	Duodenal	139.1 ± 2.6
		Jejunal	141.0 ± 5.7
21 days old			
Normoxic (12)	44.7 ± 0.	Duodenal	144.6 ± 3.0
		Jejunal	143.7 ± 4.4
14 day post-hypoxia (12)	35.6 ± 0.6*	Duodenal	141.6 ± 7.8
		Jejunal	144.1 ± 2.6
28 days old			
Normoxic (8)	73.0 ± 2.0	Duodenal	161.8 ± 1.8
		Jejunal	169.5 ± 6.7
21 day post-hypoxia (8)	60.1 ± 1.3*	Duodenal	166.3 ± 5.0
		Jejunal	167.5 ± 6.0

Note. All values are mean ± SE. (n) is the number of animals used. * Indicates values significantly different from corresponding control values with $P \leq 0.05$. Hypoxic groups were exposed to hypoxic environment for 7 days (from 0 to 7 days of age) and were then returned to normoxic environment for an additional 7, 14, or 21 days (7 day, 14 day, and 21 day posthypoxia) before sacrifice, corresponding to 14, 21, and 28 days of age. The number of litters was three from each group of 21-day-old rats and two from each group of 14- and 28-day-old rats.

small intestinal mucosa (Table III). Like sucrase and maltase, leucine aminopeptidase was unchanged at 7 days posthypoxia, but was lower in 14 days posthypoxia and returned to the control levels at 21 days posthypoxia. We also determined whether other enzymes were affected. For this, we choose the cytoplasmic marker enzyme LDH by comparing their activities in the 21-day normoxic and posthypoxic groups. There was no difference in LDH activity in the duodenal segment. In contrast, a decrease in LDH activity was observed in the jejunal segment in the recovering hypoxic group (318.3 ± 3.6 units) compared with control (443.2 ± 24.3 units).

Figure 3 summarizes the effect of daily treatment of newborn rats from birth to 7 days with a low dose of dexamethasone (simulating the hypercorticosteroid condition in the hypoxic 7-day-old animals) on intestinal lactase and maltase activity. Except for lactase in the duodenal segment, treatment with the vehicle (dimethyl sulfoxide) alone resulted in no change in any of the measurements. Treatment with dexamethasone for the same duration resulted in a moderate decrease in body weight (not shown). Small intestinal mucosal protein content did not change, but maltase activity was significantly increased by dexamethasone in both duodenal and jejunal segments. Sucrase activity was not detectable in both segments in controls and vehicle-treated groups. Treatment with dexamethasone resulted in measurable sucrase activity (1.0 ± 0.4 in the duodenal and 11.1 ± 3.8 units in the jejunal segment). Lactase was increased in the jejunal segment.

Discussion

In rats, as in humans, the digestive enzymes of the small intestine are immature and undergo rapid development postnatally (6–10, 14). Characteristically, lactase is high in neonates and decreases rapidly at weaning to reach a much lower adult level. In contrast, sucrase is undetectable and maltase is low in neonates. Just before weaning, sucrase starts to appear and increases sharply together with maltase to reach high adult levels of both enzymes. Glucocorticoids play important roles in regulating the development of these intestinal mucosal enzymes in neonatal rats (14, 15). Physiologically, these mucosal enzymes are essential in digesting food for assimilation to provide the necessary nutrients for maintenance and rapid growth in this period. The present study evaluated the effect of 7 days of hypoxia on small intestinal mucosa enzyme development in neonatal (suckling) and juvenile (weaned) rats. Hypoxia induced significant increases in lactase and maltase in the 7-day-old but not in the 35-day-old (weaned) hypoxic rats. This possibly can be due to the endogenous rise in corticosterone documented previously (11–13) and is in agreement with findings that exogenous administered glucocorticoids induce the precocious development of intestinal digestive enzymes in suckling animals (14).

Developmentally, there is a rapid growth of the small intestine and increases in mucosal enzymes before weaning (21 days) (6–10). Hypoxia during this critical stage of ontogeny might alter the normal pattern of development of the small intestine. The present study indicated that hypoxia also affected the subsequent maturation of the mucosal enzymes. Although intestinal mucosal enzyme activities were similar 7 days after recovery, mucosal maltase, sucrase, and leucine aminopeptidase in the duodenal segment remained

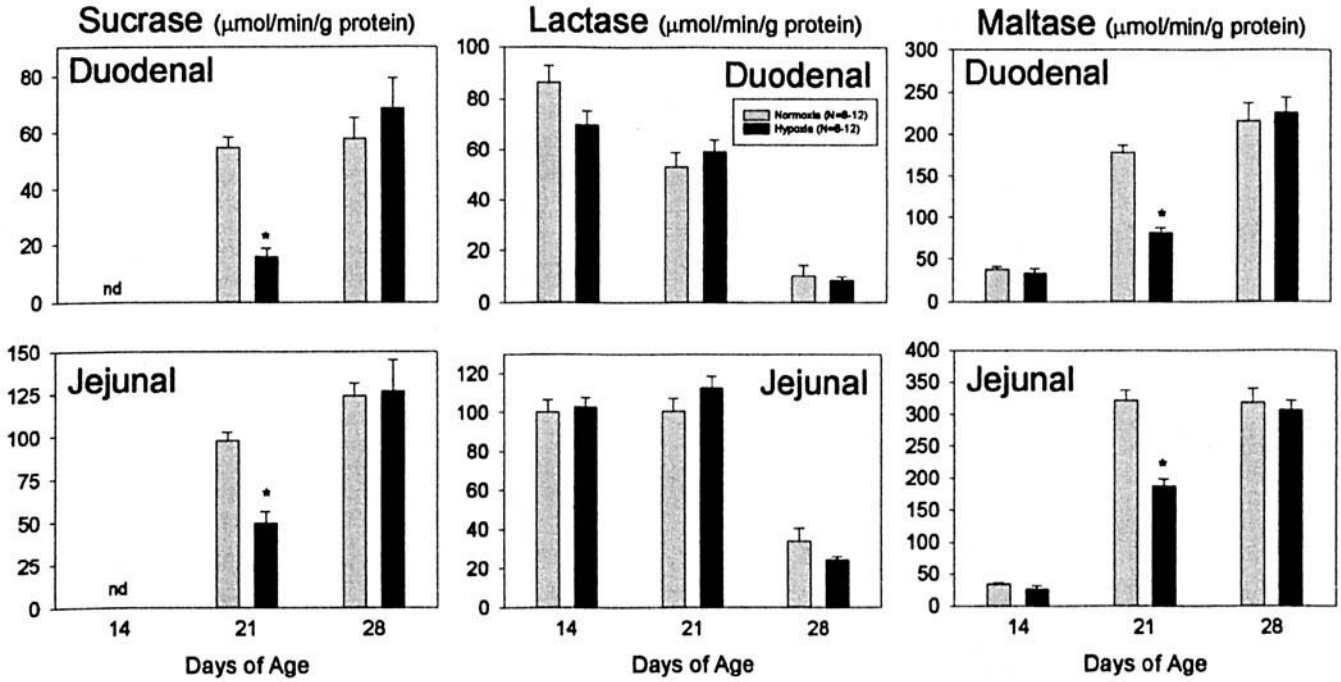

Figure 2. Effects of prior neonatal hypoxia on recovery of sucrase, lactase, and maltase development in the small intestine. Hypoxic groups were exposed to hypoxic environment for 7 days (from 0 to 7 days of age) and were then returned to a normoxic environment for an additional 7, 14, or 21 days (7 days, 14 days, and 21 days posthypoxia) before sacrifice, corresponding to 14, 21, and 28 days of age. Vertical lines above bars represent SE of the means. nd, not detectable. * Indicates that values differ significantly from corresponding control values ($P \leq 0.05$). There were two litters and eight animals in each of the 14 and 28 days group and 12 animals from three litters in each of the 21 days group.

Table III. Changes in Leucine Aminopeptidase Activity in 7-Day Hypoxic Rats after Return to Normoxic Environment

Age	Intestinal segment	Leucine aminopeptidase (nmol/min/mg protein)
14 days old		
Normoxic (8)	Duodenal	32.3 ± 3.0
	Jejunal	43.9 ± 3.3
7 day posthypoxic (8)	Duodenal	30.3 ± 2.0
	Jejunal	44.2 ± 4.0
21 days old		
Normoxic (8)	Duodenal	66.6 ± 2.7
	Jejunal	101.0 ± 5.3
14 day posthypoxic (8)	Duodenal	48.0 ± 3.1*
	Jejunal	76.7 ± 5.5*
28 days old		
Normoxic (8)	Duodenal	44.8 ± 5.0
	Jejunal	79.1 ± 4.7
21 day posthypoxic (8)	Duodenal	44.4 ± 4.7
	Jejunal	75.7 ± 4.5

Note. All values are mean ± SE. (n) is the number of animals used. * Indicates values significantly different from corresponding control values with $P \leq 0.05$. Hypoxic rats were exposed to hypoxic environment for 7 days (from 0 to 7 days of age) and were then returned to normoxic environment for an additional 7, 14, or 21 days (7 day, 14 day, and 21 day post-hypoxia) before sacrifice, corresponding to 14, 21, and 28 days of age.

lower than control age-matched groups 2 weeks after recovery from hypoxia. It is not until 3 weeks after recovery that these enzymes returned to the levels found in the control group. Therefore, there is an attenuation of the development of disaccharidase, particularly that of maltase and sucrase in the small intestine of the hypoxic rats.

The cytoplasmic marker enzyme LDH was spared in the duodenal segment and the relative decrease of LDH in the jejunal segment (25% less) was less severe than the relative decreases of sucrase (50%–80% less) or maltase (50%–60% less). This indicated that the effect of hypoxia apparently was more specific to digestive enzymes.

Within this same recovery period, the hypoxic group did not exhibit catch-up growth (weight gain per week). Catch-up development of the small intestinal enzymes was not observed until 3 weeks into recovery. Even at that time, the body weight of the hypoxic group still lagged behind that of the control group. This has important implications because hypoxia is recognized as one of the most common neonatal syndromes. Hypoxia at birth due to prematurity, cardiac defects, trauma, blood loss, or obstruction of airway accounts for varying durations of hypoxia in infants (22) and accounts for considerable morbidity and mortality (1, 2). Although the primary concerns of neonatal hypoxia are the detrimental effects on neurological, cardiopulmonary, and renal changes, the observed delay in small intestinal mucosal enzyme together with a recently documented delay in pancreatic exocrine enzyme development (23) might play a part in the nutritional adaptation of the hypoxic infants, particularly during the recovery phase. The small intestine and the exocrine pancreas normally provide most of the key

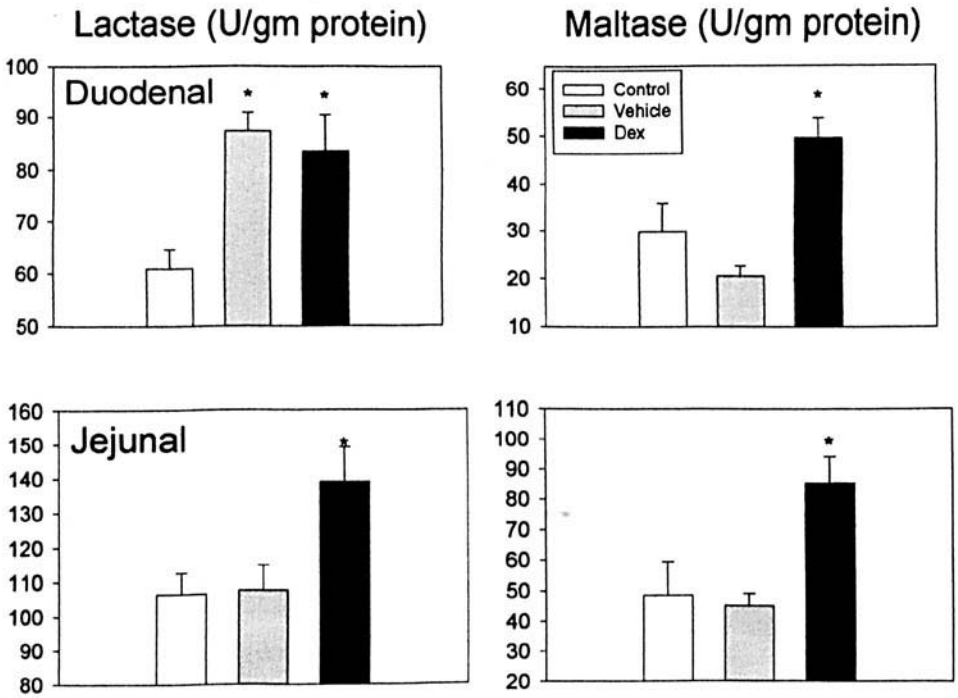

Figure 3. Effects of low-dose of dexamethasone treatment of rats from birth to 6 days of age on mucosal lactase and maltase activity in the proximal and distal segments of the small intestine from 7-day-old rats. Newborn rats were given dexamethasone (Dex, 13 ng/gm body weight) or vehicle (same quantity of dimethyl sulfoxide, DMSO) daily from 0 to 6 days of age. Treated rats were sacrificed at 7 days of age together with age-matched controls that received no injection. Vertical lines above bars represent SE of the means. * Indicates that values significantly different from corresponding values in untreated controls with $P \leq 0.05$. There were six animals in each group.

hydrolytic enzymes for lipid, protein, and carbohydrate digestion. All of these nutrients are essential as fuels and building blocks for essential metabolites in growth and repair. A delay in the maturation of these enzymes might have profound effects on the nutritional health of the infant in this critical period of growth.

Interestingly, the 7-day posthypoxic rats (at 14 days of age) showed mucosal enzymes very similar to the controls. Yet some differences in these measurements were evident 7 days after. One explanation is that increases of maltase and lactase, caused by increase in endogenous corticosterone in the hypoxic group at 7 days of age, is a transient phenomenon. Upon release from hypoxia, the hormonal effect dissipates and the enzymes return to their original levels. From 14 to 21 days of age, the small intestine undergoes rapid transformation (6–10). Prior hypoxic condition then manifested its effects by upsetting the genetic programming and the timing of differentiation of mucosal cells thus leading to a delayed appearance of mucosal enzymes in the 21-day-old animals (14 days posthypoxic).

Although the exact mechanism for the effects of hypoxia on intestinal enzyme development is not known, we speculated that hypoxia might act through modulation of the endocrine system. One observation that is relevant to the present study is that of an increase in plasma corticosterone during neonatal hypoxia as reported previously (11–13). Glucocorticoids have been known to have important function in modulating the ontogeny of many enzyme systems, notably in the exocrine pancreas and the gastrointestinal tract (7). In the present study, we attempt to simulate increases in plasma glucocorticoids as observed in hypoxia by daily administration of a low dose of dexamethasone (a synthetic analog of glucocorticoid with a longer biological half-life and a higher potency than corticosterone) for the same duration as the hypoxic treatment in newborn rats from birth to 7 days of age. Dexamethasone treatment induced the appearance of sucrase and an increase in maltase and lactase activity. As it is impossible to exactly duplicate the hyperglucocorticoidism in hypoxia that is persistent and physiological, we have to interpret these observations with caution. Still, these results are somewhat different from those observed with hypoxia because the latter led to increases in maltase and lactase, but no induction in sucrase. Thus, the increase in glucocorticoids resulting from hypoxic treatment might have contributed in part to the observed decrease in body growth. However, hyperglucocorticoidism could only account for the increase in maltase activity, but not for the response of other enzymes in hypoxia.

Other possible mechanisms were also considered. One is a change in the behavior of the hypoxic dams, but we have not seen any obvious changes in maternal behavior. As for other factors, we have shown that pups from hypoxic and normoxic groups had similar serum concentrations of 25-hydroxyvitamin D, osteocalcin, total and ionized calcium, and total phosphorous. These serve as indirect evidence for the absence of malabsorption at least for these nutrients in the hypoxic animals (24). We have also analyzed milk fat composition from hypoxic dams and it is not appreciably altered (11). Because we have not directly measured milk production, we cannot completely eliminate it as a confounding variable. However, the growth rate of hypoxic pups tends to parallel that of the normoxic controls after an initial dramatic decrease (13). Taken together, these suggest that alterations in milk production or nutrient absorption is not a major cause of the phenomenon observed.

In conclusion, the present study showed that hypoxia in

neonatal rats caused a delay in the maturation of small intestinal enzymes. Hypoxic conditions led to a delay in marker mucosal digestive enzyme maturation that, in turn, might cause a delay in catch-up growth. Increases in plasma glucocorticoids due to hypoxia are probably not a major mechanism in the delay in small intestinal enzyme maturation.

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